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REPRODUCTIVE BIOLOGY OF NAIADS IN THE UPPER BLUE NILE

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INTRODUCTION

The freshwater mussels of the order Unionida (naiads) are unique because their larvae, which are brooded in marsupial demibranchs, are obligate parasites on fish hosts. Their evolution has been influenced by a long and complex history that ranges over the entire planet and extends over 200 million years (Watters, 2001). However, their systematic relationships (e.g., monophyletic versus polyphyletic origin, number of families and species etc.) are still heavily debated (e.g., Graf & Cummings, 2006, 2007; Hoeh et al., 2009; Whelan et al., 2011), particularly because of two different types of larvae, glochidium and lasidium.

The components of the naiad assemblage in the Nile have evolutionary ties to a wide range of taxa around the world. Three families occur in the Nile system, Unionidae, Iridinidae and Etheriidae (Graf & Cummings, 2007). Furthermore, the Nile system is the only area where palaearctic and Afro-tropical molluscs overlap. During the Quaternary cold phases, palaearctic species extended as far south as Lake Turkana. Now they are separated from Afro-tropical species by the Sahara and occur mainly in the coastal areas of northwest Africa. The only river crossing the Sahara from tropical Africa north to the Mediterranean Sea is the Nile, and palaearctic species still extend as far south as to its headwaters (Van Damme, 1984).

For most Nile species, data on reproductive traits would be necessary to detect their relation to other taxa around the world. In biogeographical respect, this naiad fauna is marked by:

(a) a high rate of endemism to the Nile,
(b) the southern extent of Palaearctic species, and
(c) Afro-tropical species.

The three naiad species surveyed here – discussed by Van Damme, 1984, and Graf & Cummings, 2007 – are representatives of these three categories:

Unio abyssinicus (Martens, 1866), is a Blue Nile endemic of an otherwise mainly European genus. During the Pleistocene, it was widely distributed in the Nile system up to northern Egypt, but it disappeared from most areas during the early or middle Holocene (10,000 – 5,000 B.C.), and it now occurs in Lake Tana and the upper Blue Nile (Van Damme, 1984; Graf & Cummings, 2007). The reproductive biology of this species has been unknown.

Unio mancus (Lamarck, 1819) (synonym: *U. elongatulus* Rossmaessler, 1835) is distributed around the Mediterranean Sea (Araujo et al., 2005). It is the common *Unio* of southern France, northeastern, central and southern Spain, Italy and parts of the Middle East (Nagel et al., 1998, Araujo et al., 2005). Two African subspecies are geographically isolated. According to Van Damme (1984), the one occuring in Lake Tana and the Blue Nile is considered *U. mancus dembeae* (Reeve, 1865). It is the only palaearctic naiad reaching the Nile (Graf & Cummings, 2007; Van Damme, 1984). Reproductive data are available for some populations of *U. mancus* on the Iberian Peninsula (Araujo et al., 2005) and Italy (Castagnalo, 1977), but not for *U. mancus dembeae*.

Etheria elliptica (Lamarck, 1807), the cementing Nile Oyster, is widespread in tropical Africa, where it is found in the basins of the Nile, Niger, Congo and even in Madagascar. The northern limits of its range are the Senegal and Niger Rivers and Lake Tana. The oldest fossils date from the Early Miocene; however, its occurence in Madagascar suggests a greater phylogenetic age (Yonge, 1962; Van Damme, 1984). Its systematic position and its relationship to the other two cementing naiad species in South America and South India is unclear (Hoeh et al., 2009; Bogan & Hoeh, 2000), and little is known about its life history. In particular, data on its reproductive biology has not been available. Some subspecies or races have been described, which are very different in size and shell morphology (Yonge, 1962). My results indicate also different modes of reproduction. Therefore, in this paper I use just the generic name *Etheria.*

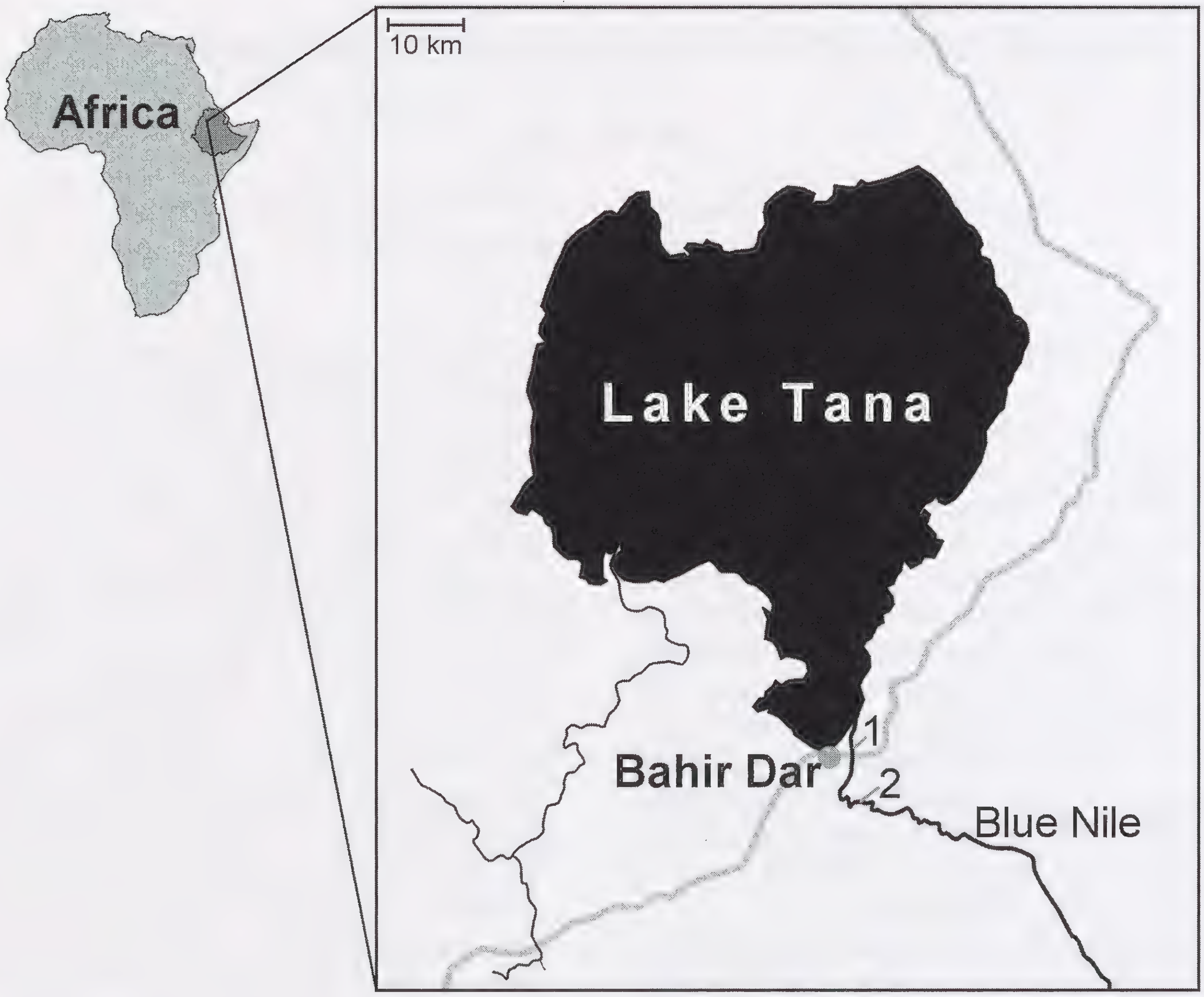


FIG. 1. The study area Lake Tana and the origin of the Blue Nile in the Ethiopian Highland; 1 = Sample site 2009, 2 = Sample site 2010.

METHODS

The investigations were conducted in Bahir Dar, Ethiopia, at the origin of the Blue Nile (Fig. 1), which is the outlet of Lake Tana at an altitude of approximately 1,700 m. Mussels were collected by fishermen, in the first study period (January – February 2009) from the Blue Nile close to Lake Tana, in the second study period (March 2010) approximately 10 km downstream of Bahir Dar (Fig. 1). During this second period, only *Etheria* was collected.

TABLE 1. Reproductive status of 46 *Unio abyssinicus* individuals in the upper Blue Nile (January 2009).

Shell length (mm)	30–40	40–50	50–60	> 60	sum
males	1	5	13	2	21
females		6	14	5	25
gravid		3	9	2	14

All specimens of *Unio* were preserved as a whole in 100% ethanol. In Germany, they were examined for gravidity, and sex was determined by making an incision in the visceral mass dorsal to the foot and analysing the smear under the microscope. Oocytes and spermatozoa could be easily recognized.

Etheria was dissected in Ethiopia, and the gills were examined using a binocular microscope for gravidity. Pieces of gravid gills were fixed in ethanol for further examination in Germany.

The measurements of egg or larval sizes were performed using an Zeiss Axioskope with the Optimas programme. Some glochidia were cleared with KOH to show the shell structures.

RESULTS

Unio abyssinicus

We collected 46 specimens. They were dioecious, with a nearly equal sex ratio (Table 1).

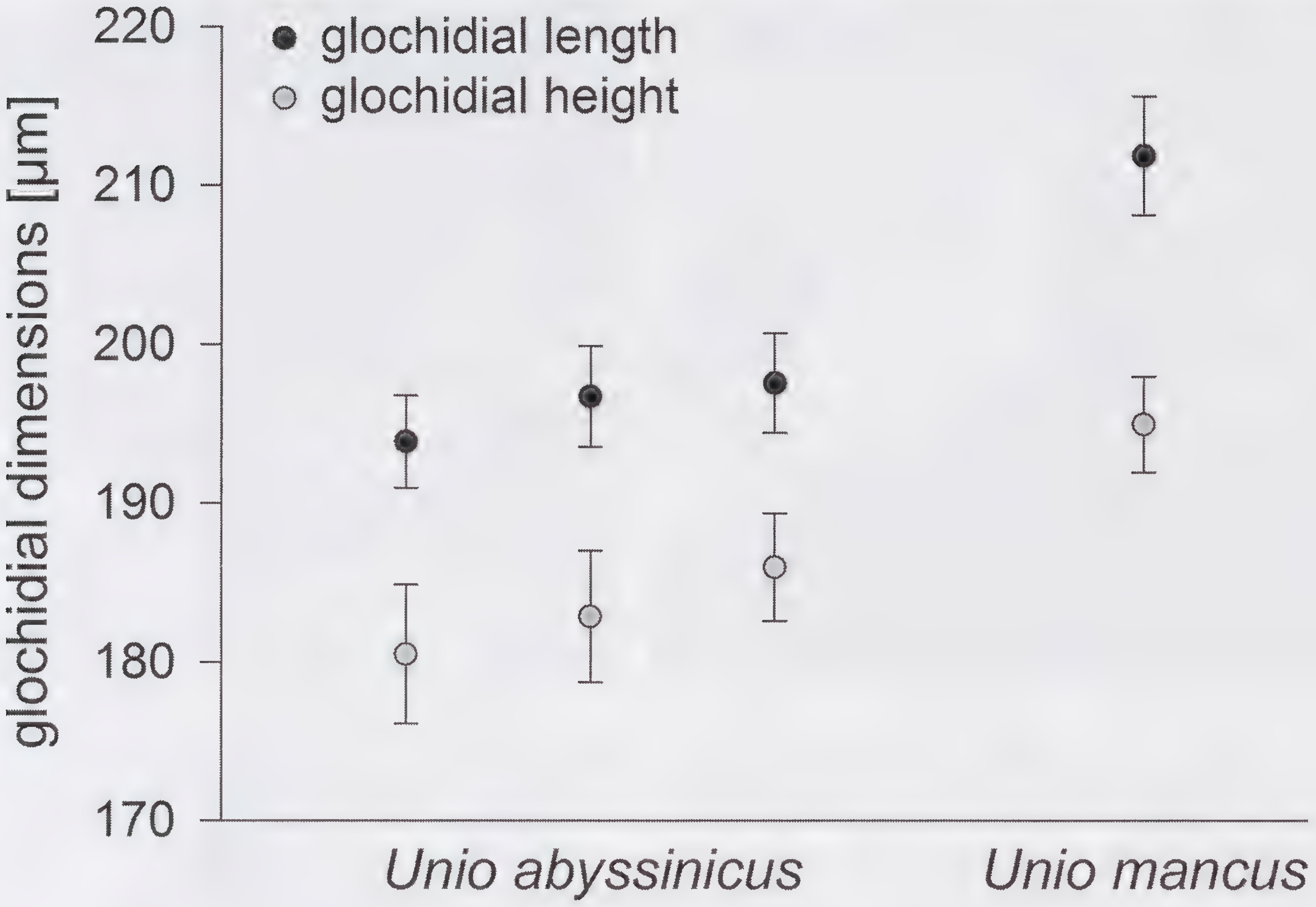
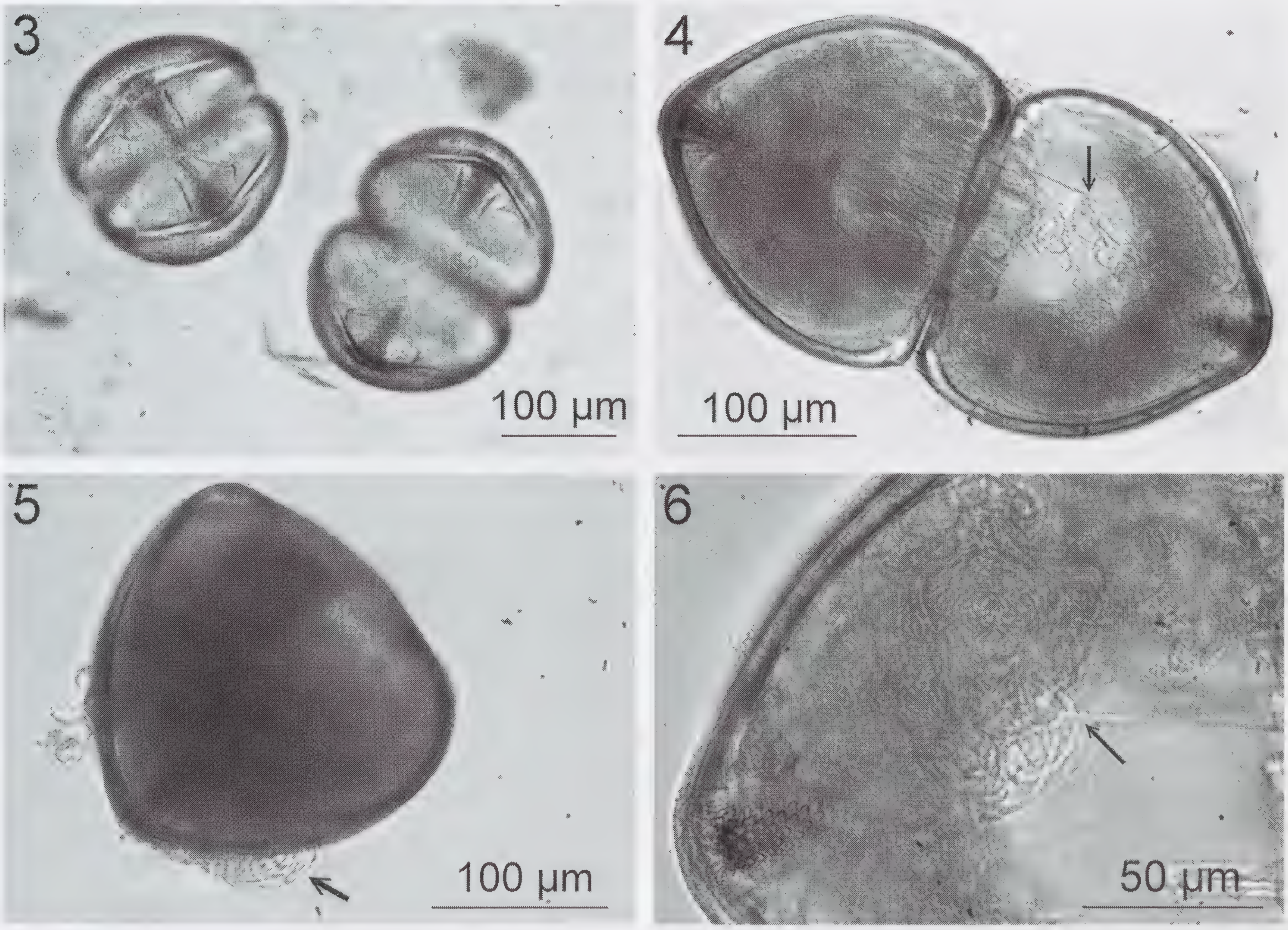
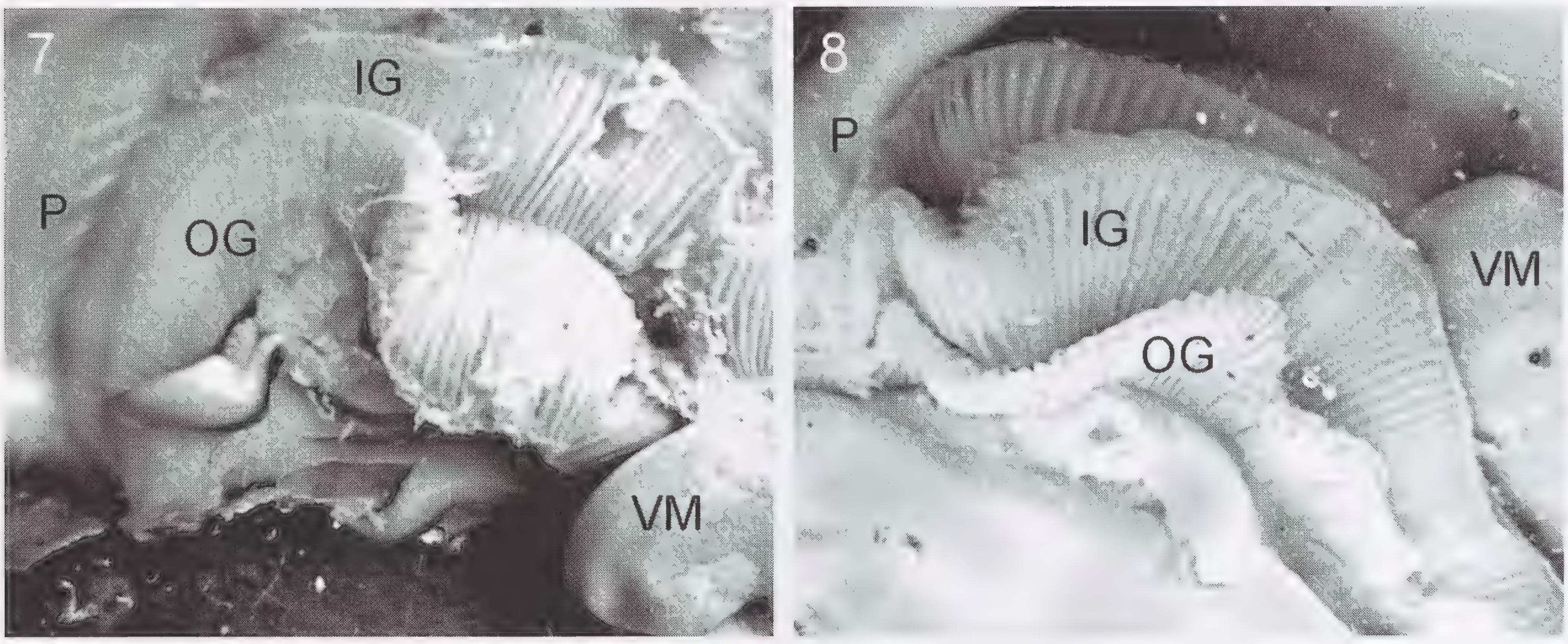


FIG. 2. Glochidial shell length and height (mean ± sd) of three *U. abyssinicus* and one *U. mancus* females from the Blue Nile (twenty measurements per female).



FIGS. 3–6. Glochidia of the genus *Unio*. FIGS. 3, 4: *U. abyssinicus*; FIGS. 5, 6: *U. mancus dembeae*. Arrows point to the larval thread.



FIGS. 7, 8. Gills of gravid *Etheria.* FIG. 7: tetrabranchous; FIG. 8: endobranchous (IG = inner gill, OG = outer gill, P = posterior end, VM = visceral mass). Note the marked swelling of the posterior endobranchous gill in Fig. 8.

Of the 25 females, 14 had been gravid in the outer gills with various developmental stages of offspring. Hardly any unfertilized eggs were observed. Three specimens contained mature glochidia. Their shape is triangular with a mean shell length of 196 µm (Fig. 2), with prominent hooks and a larval thread (Figs. 3, 4).

Unio mancus dembeae

The only specimen we found contained mature glochidia and unfertilized eggs in the outer gills. However, the percentage of glochidia was very low, namely 21% (n = 257 eggs analysed). Glochidia are triangular, and like *U. abyssinicus* a little bit higher than long (Fig. 2), with hooks and a larval thread (Figs. 5, 6).

Etheria

In the first study period, out of 55 specimens with a shell length between 5 and 10 cm, 28 were gravid. All females were tetrabranchous, meaning the eggs are brooded in all four gills (Fig. 7). During the second study period, 11 gravid females were tetrabranchous and 5 were endobranchous (Fig. 8). Among individuals, the stage of offspring development was somewhat different. During the first study period, only one specimen contained larvae at an early developmental stage. They were lasidia, with an undivided spherical cuticula of 60 µm diameter and two prominent ciliated anterior lobuli (Figs. 9, 10). The structures at the posterior end are apparently hooks (Fig. 11), as described for all lasidia.

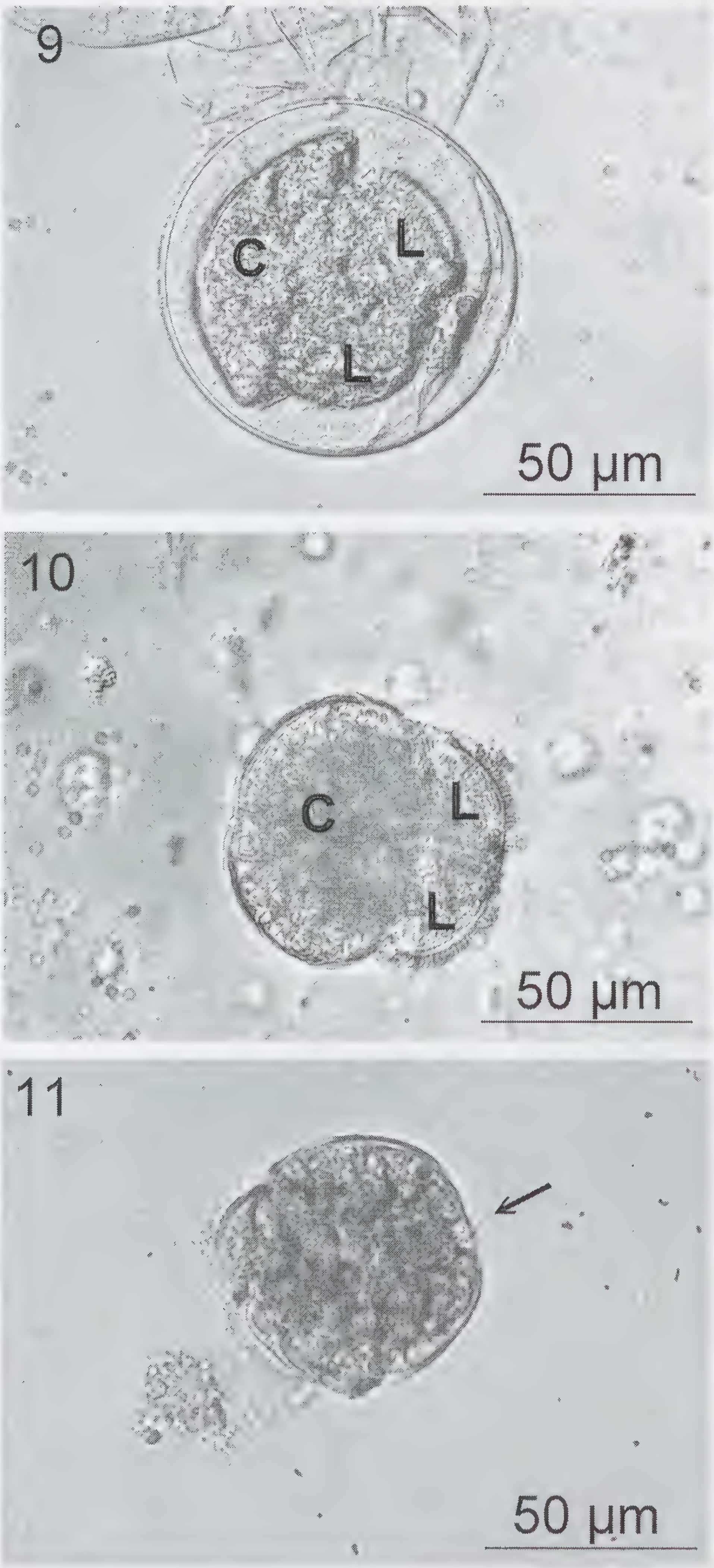
During the second study period, all tetrabranchous females contained just eggs, whereas in some endobranchous females lasidia were found. Egg size was similar in both groups (Fig. 12). The lasidia of the endobranchous specimen are marked by an anterior organ of unknown function (Fig. 13).

DISCUSSION

Reproduction of *Unio*

The genus *Unio* is widely distributed in the palaearctic. The study of its taxonomy is still an ongoing task (Araujo et al., 2005; Khalloufi et al., 2011). However, within this group, a pretty clear and uniform pattern emerges with respect to the reproductive strategy: all investigated species have been found to be largely of separate sex, with an equal ratio, and the glochidia are brooded in the outer gills (Hochwald, 1988; Araujo et al., 2005). There is a critical population density below which fertilization of eggs declines (Hochwald, 1988). During the reproductive season of some months, there are several spawnings and refillings of marsupia, which are not synchronized within a population (Hochwald, 2001). The hooked glochidia are triangular, with a long larval thread (reviewed in Reis & Araujo, 2009), and their size is intermediate among glochidia (Bauer, 1994).

All these results about *Unio* are from palaearctic and northwest African species. However, they are confirmed by the data from the two *Unio* species in the Blue Nile.



FIGS. 9–11. Lasidia of a tetrabranchous *Etheria* (2009). FIG. 9: Frontal view of a larva still encapsulated; FIG. 10: Free lasidium from above (C = cuticula, L = anterior ciliated lobuli); FIG. 11: Free lasidium. The arrow points to the posterior hooks.

Unio abyssinicus is dioecious (Table 1). Only about half of the females were gravid, arguing for an extended reproductive season probably with several spawnings. Furthermore, the considerably different developmental stages of eggs indicate that reproduction is not synchronized within the population. Glochidial size is at the lowest limit thus far reported for *Unio* (Fig. 2).

The only individual of *Unio mancus dembeae* contained mature glochidia in the outer demibranchs; however, most of the eggs (79 %) had not been fertilized. With a shell length of approximately 212 µm (Fig. 2), its glochidia are smaller than the glochidia found in Iberian and Italian *U. mancus* populations (217–224 µm; reviewed in Araujo et al., 2005). It was the rarest naiad in the surveyed area. Together with the high rate of unfertilized eggs, this argues that *U. mancus* is also dioecious in the Blue Nile, as reported in Iberian populations (Araujo et al., 2005) and that the rate of fertilization declines below a critical population density, as reported for European *U. crassus* Retzius, 1788, populations (Hochwald, 1988). However, whereas in Europe it is a summer breeding species (Castagnalo, 1977; Araujo et al., 2005), in the Blue Nile reproduction occurs at least during January and February. The different breeding season, in connection with the comparatively small glochidium, might render *U. mancus dembeae* a potential candidate for a new *Unio* species if more data are available.

Reproduction of *Etheria*

A serious contradiction to published data concerns the mode of brooding of *Etheria*. Thus far the Etherioidea have been reported to be endobranchous, and this trait is considered to be a systematic characteristic of this superfamily (Graf & Cummings, 2006; Walker et al., 2006; Varjabedian, 2006; Herrmann, 1995; Artaega, 1994; Heard & Vail, 1976). But during the first study period, I found all gravid individuals of *Etheria* with eggs in all four demibranchs (tetrabranchous; Fig. 7).

During the second study period, where the mussels had been collected approximately 10 km downstream, I found 16 gravid females: 11 were tetrabranchous, 5 were clearly endobranchous (Fig. 8). Whereas egg size was similar (Fig. 12), only the endobranchous females contained ripe larvae. So far, the Etheriidae are considered to contain only one species with a large distribution area (Bogan, 2008). However, many forms have been described (Yonge, 1962), and my results indicate different reproductive modes. Therefore, I think that the genus *Etheria* comprises several species.

In 2009, out of 57 *Etheria* individuals examined, 28 were found to be gravid. This argues for an equal distribution of separate sexes, confirming the results of Ampofo-Yeboah et al. (2009) about *Etheria* in Ghana. The same condition has been found among other African

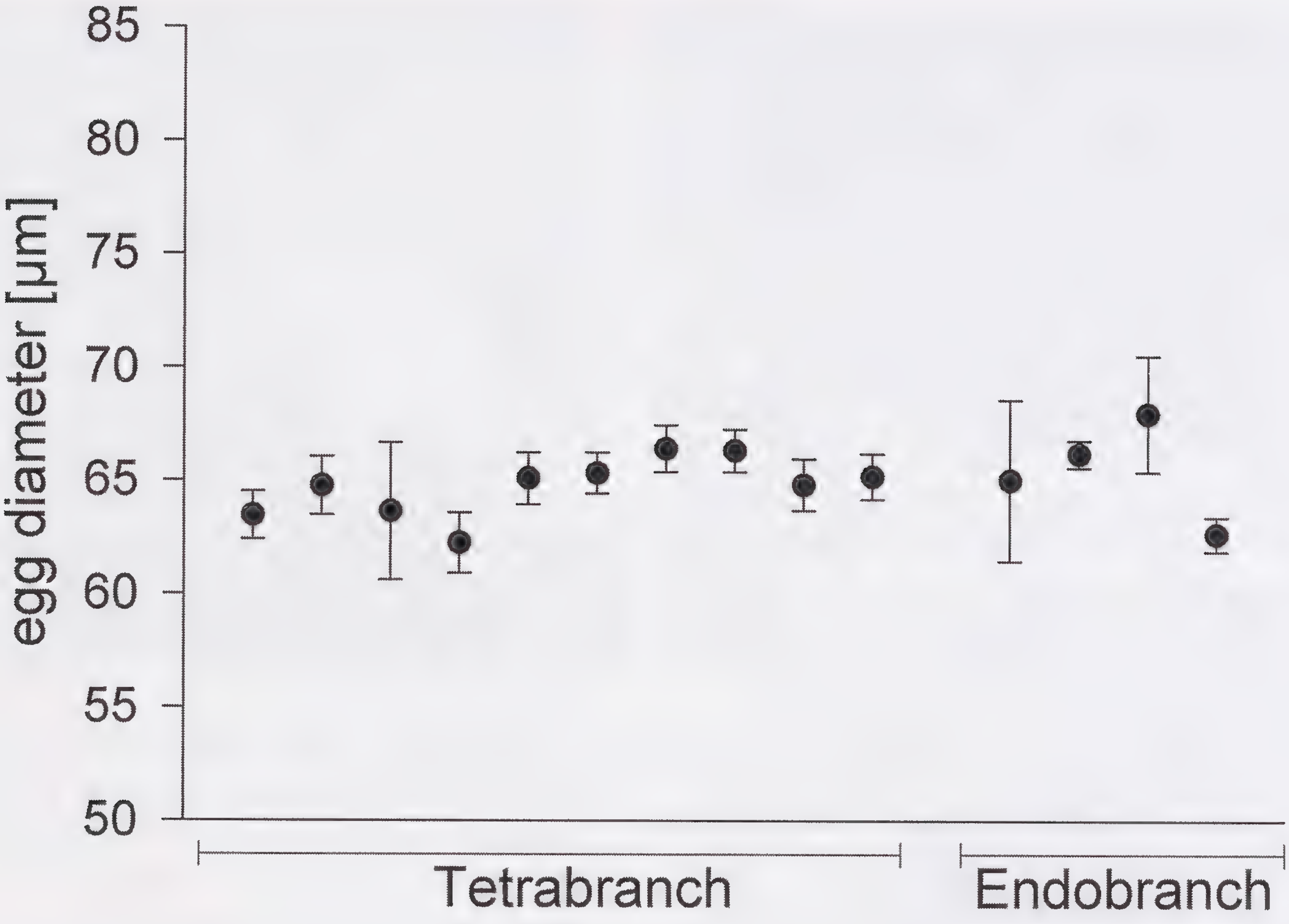


FIG. 12. Egg size (mean ± sd) of tetrabranchous and endobranchous *Etheria* collected 2010.

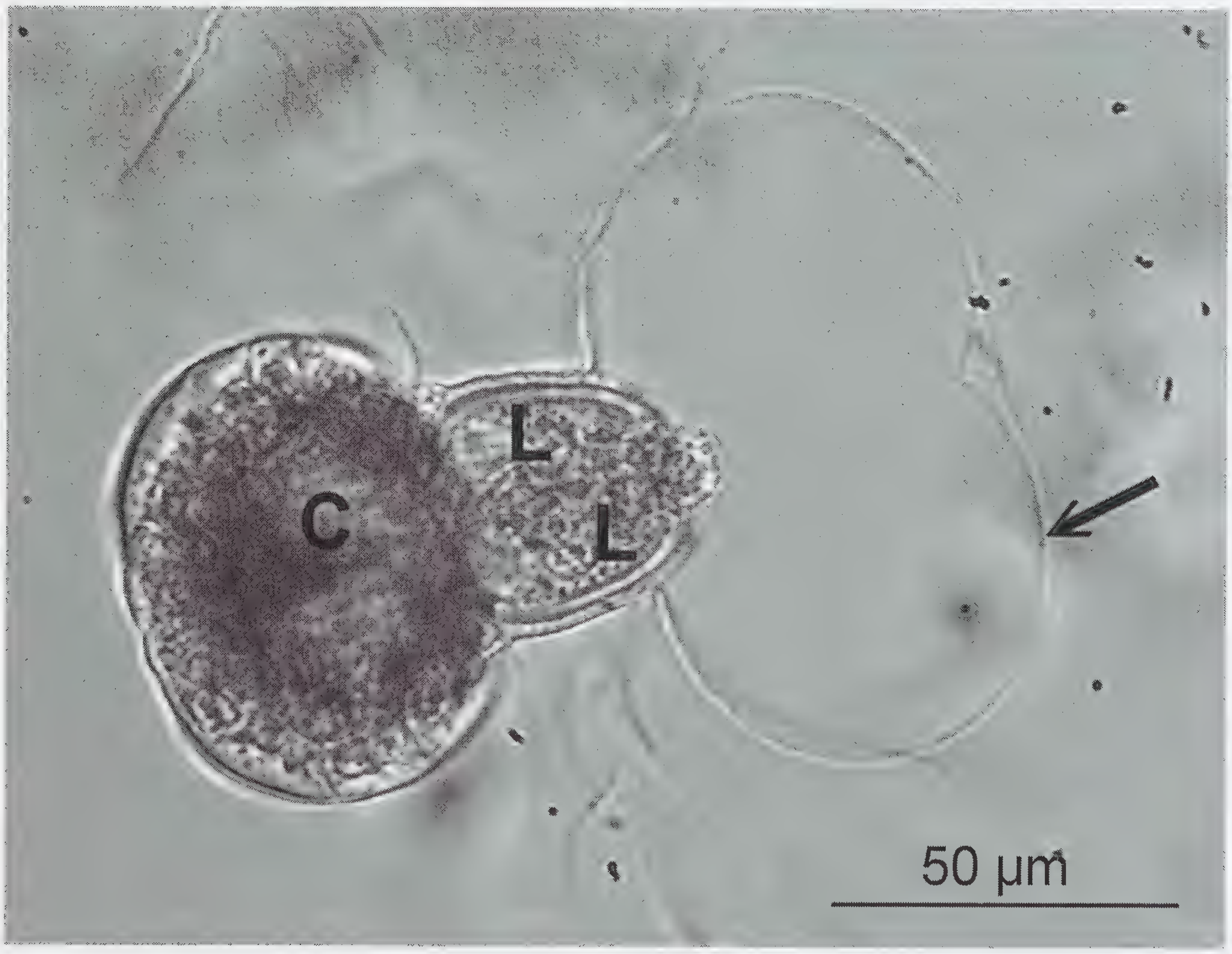


FIG. 13. Lasidium of an endobranchous *Etheria* (C = cuticula, L = anterior lobuli). The arrow points to an “anterior organ”, which might be related to the “ribbon like organ” of South American lasidia.

Etherioidea collected in the Nile River in Egypt, namely *Mutela dubia nilotica Crosse, 1881, M. bourguignati* Bourguignat, 1885, and *Chambardia rubens* (Savigny, 1827) (Falk, 1995; Walker et al., 2006). However, *M. rostrata* (Rang, 1835) is typically hermaphroditic. In South America, two Mycetopodidae were found to be hermaphroditic (Walker et al., 2006).

Since apparently all females were gravid during my first study period, reproduction seems to be largely synchronized, and the reproductive season in the upper Blue Nile is during low water level in January and February, confirming the results of Heard & Vail (1976) from Zaire.

Compared to the few records about the size of lasidia (Fryer, 1961; Parodiz & Bonetto, 1963; Varjabedian, 2006), the one of *Etheria* is among the smallest (60 µm, Figs. 9–11, 13).

A larval thread, as in the iridinid *M. bourguignati* was not found. However, the larvae of endobranchous females (Fig. 13) develop a structure that might be related to the anterior ribbon like organ of attachment of South American mycetopodid lasidia (Parodiz & Bonetto, 1963; Bonetto, 1997). From tetrabranchous females, I got only larvae at an early developmental stage (Figs. 9–11). Therefore, it cannot be excluded that also these larvae develop this organ later. A prominent ribbon like organ is also present at the lasidium of *Acostaea rivoli* (Deshayes, 1827), the South American "freshwater oyster" (Artaega, 1994).

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LITERATURE CITED

AMPOFO-YEBOAH, A., M. OWUSU-FRIMPONG & K. YANKSON, 2009, Gonad development in the freshwater oyster *Etheria elliptica* in northern Ghana. *African Journal of Aquatic Science*, 34(2): 195–200.

ARAUJO, R., I. GOMEZ & A. MACHORDOM, 2005, The identity and biology of *Unio mancus* Lamarck, 1819 in the Iberian Peninsula. *Journal of Molluscan Studies*, 71: 25–31.

ARTAEGA, S. E., 1994, El lasidium de *Acostaea rivoli* y su importancia paraubicacioin taxonomica de esta especies. *Boletin Ecotropica: Ecosystemas Tropicales*, 27: 1–9.

BAUER, G., 1994, The adaptive value of offspring size among freshwater mussels. *Journal of Animal Ecology*, 63/4: 933–944.

BOGAN, A., 2008, Global diversity of freshwater mussels in freshwater. *Hydrobiologia*, 595: 139–147.

BOGAN, A. E. & W. R. HOEH, 2000, On becoming cemented: evolutionary relationships among the genera in the freshwater bivalve family Etheriidae (Bivalvia: Unionoida). Pp. 159–168, in: E. M. HARPER, J. D. TAYLOR & J. A. CRAME, eds., *The evolutionary biology of the Bivalvia.* Geological Society, London, vii + 494 pp.

BONETTO, A. A., 1997, Las «ostras de aqua dulce». Su taxonomia y distribution geographicaa en el conjunto de las naiades del mundo. *Biociencias*, 5: 113–142.

CASTAGNALO, L., 1977, Ciclo biologico riproduttivo di *Anodonta cygnea* e *Unio elongatulus* nel po di tolle. *Rivista di Hidrobiologia*, 16: 3–14.

FALK, L., 1995, *Das ektoparasitische Larvenstadium der afrikanischen Süßwassermuschel Mutela burguignati*. Ph.D. thesis, Tierärztliche Hochschule Hannover.

FRYER, G., 1961, The developmental history of *Mutela burguignati*. *Philosophical Transactions of the Royal Society of London*, 244: 259–298.

GRAF, D. L. & K. S. CUMMINGS, 2006, Palaeoheterodont diversity (Mollusca: Trigonoida + Unionoida): what we know and what we wish we knew about freshwater mussel evolution. *Zoological Journal of the Linnean Society*, 148: 343–394.

GRAF, D. L. & K. S. CUMMINGS, 2007, Preliminary review of the freshwater mussels of northern Africa, with an emphasis on the Nile. *Journal of the Egyptian German Society of Zoology*, 53D: 89–118.

HEARD, W. H. & V. A. VAIL, 1976, Anatomical systematics of *Etheria elliptica*. *Malacological Review*, 9: 15–24.

HERRMANN, F., 1995, *Untersuchungen zum Lebenszyklus der afrikanischen Süßwassermuschel Mutela burguignati* Burguignat 1885. Ph.D. thesis, University Hannover.

HOCHWALD, S., 1988, *Untersuchungen zur Populationsbiologie und Fortpflanzungsbiologie der Bachmuschel Unio crassus*. M.S. thesis, University of Bayreuth.

HOCHWALD, S., 2001, Plasticity of life-history traits in *Unio crassus*. Pp. 127–141, in: G. BAUER & K. WAECHTLER, eds., *Ecology and evolution of the freshwater mussels Unionoida*. Ecological Studies, 145, Springer Publishers, Berlin, xvii + 394 pp.

HOEH, W. R., A. E. BOGAN, W. H. HEARD & E. G. CHAPMAN, 2009, Palaeoheterodont phylogeny, character evolution, diversity and phylogenetic classification: a reflection on methods and analysis. *Malacologia*, 51(2): 307–317.

KHALLOUFI, N., C. TOLEDO, A. MARCHORDOM, M. BOUMMAIZA & R. ARAUJIO, 2011, The unionids of Tunesia: taxonomy and phylogenetic relationships, with rediscription of *Unio ravoisery* and *U. deshayes*. *Journal of Molluscan Studies*, 77(2): 103–115.

NAGEL, K. O., O. BADINO & G. CELEBRANO, 1998, Systematics of European naiads: a review and some new aspects. *Malacological review*, Suppl. 7: 83–104.

PARODIZ, J. J. & A. A. BONETTO, 1963, Taxonomy and zoogeographic relationships of the South American naiads. *Malacologia*, 1: 179–213.

REIS, J. & R. ARAUJO, 2009, Rediscription of *Unio tumidiformis*, an endemism from the southwestern Iberian Peninsula. *Journal of Natural History*, 43: 1929–1945.

VAN DAMME, D., 1984, The freshwater Mollusca of northern Africa: distribution, biogeography and palaeoecology. *Developments in Hydrobiology*, 25: 162 pp.

VARJABEDIAN, K. G., 2006, The early development of two unionid bivalves from the River Nile, Egypt. *Journal of the Egyptian German Society of Zoology*, 50D: 139–152.

WALKER, J. M., A. E. BOGAN, K. GARO, G. N. SOLIMAN & W. R. HOEH, 2006, Hermaphroditism in the Iridinidae (Bivalvia; Etherioidea). *Journal of Molluscan Studies*, 72: 216–217.

WATTERS, G. T., 2001, The evolution of the Unionacea in North America, and its implications for the worldwide fauna. Pp. 281–307, in: G. BAUER & K. WAECHTLER, eds., *Ecology and evolution of the freshwater mussels Unionoida*. Ecological Studies, 145, Springer Publishers, Berlin, xvii + 394 pp.

WHELAN, N. V., A. J. GENEVA & D. L. GRAF, 2011, Molecular phylogenetic analisys of tropical freshwar mussels resolves the position of *Coelatura* and supports a monophyletic Unionidae. *Molecular Phylogenetics and Evolution*, 61(2): 504–514.

YONGE, C. M., 1962, On *Etheria elliptica* Lam. and the course of evolution, including assumption of monomyarianism in the family Etheriidae. *Philosophical Transaction of the Royal Society London*, Series B, 244: 423–458.

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